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Review

The possible role of ammonia toxicity on the exposure, deposition, retention, and the bioavailability of nicotine during smoking ☆

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ABSTRACT

A complete and rigorous review is presented of the possible effect(s) of ammonia on the exposure, deposition and retention of nicotine during smoking and the bioavailability of nicotine to the smoker. There are no toxicological data in humans regarding ammonia exposure within the context of tobacco smoke. Extrapolation from occupational exposure of ammonia to smoking in humans suggests minimal, non-toxicological effects, if any. No direct study has examined the effect of the ammonia on the total rate or amount of nicotine reaching the arterial bloodstream or brains of smokers. Machine-smoking methods have been reported which accurately quantify >99% of the nicotine in mainstream (MS) smoke for a wide variety of commercial and test cigarettes, including a series of experimental cigarettes having a range in MS smoke ammonia yields using the US Federal Trade Commission (FTC) protocol. However, the actual exposure of nicotine to smokers depends on their own smoking behavior. The nicotine ring system is relatively thermally stable. Protonated nicotine forms nicotine which evaporates before the nicotine ring system decomposes. The experimental data indicate that neither nicotine transfer from tobacco to MS smoke nor nicotine bioavailability to the smoker increases with an increase in any of the following properties: tobacco soluble ammonia, MS smoke ammonia, "tobacco pH" or "smoke pH" at levels found in commercial cigarettes. Gas phase nicotine deposits primarily in the mouth and upper respiratory tract. To the extent that ammonia increases the deposition of nicotine in the buccal cavity and upper respiratory tract during smoking, the total rate and amount of nicotine into the arterial bloodstream and to the central nervous system will decrease. Charged nicotine analogues are actively transported in a number of tissues. This active transport system appears to be insensitive to pH and the form of nicotine in the biological milieu, suggesting that protonated nicotine may be a substrate for active transport. Neither "smoke pH" of commercial cigarettes nor "smoke pH_{eff}" nor the fraction of non-protonated nicotine in tobacco smoke particulate matter are useful, practical smoke parameters for providing understanding or predictability of nicotine bioavailability to smokers. Greater than 95% of both ammonia and nicotine are in the gas phase of environmental tobacco, and both are likely to deposit in the buccal cavity and upper respiratory tract following exposure.

Contents

Abbreviations: CFP, Cambridge filter pad is placed downstream of the cigarette in a machine-smoking method, to capture the total particulate matter; CNS, central nervous system; ETS, environmental tobacco smoke; FTC, US Federal Trade Commission; H-C, Health-Canada; ISO, International Organization for Standardization; MDPH, Massachusetts Department of Public Health; MS, mainstream i.e. the smoke stream issuing from the mouth end of a cigarette; SS, sidestream the smoke stream that issues, not from the mouth end of a cigarette, but rather from the burning end, including through the paper or filter, generally during smolder; "tar", the total particulate matter (TPM) (all the material trapped on a Cambridge filter pad during a machine-smoking protocol) minus the smoke nicotine and water content; TGA, thermogravimetric analysis; α_{fb}, the fraction of non-protonated nicotine in a specific, specified medium.

☆ Dedicated to the memory of Dr. Richard R. Baker (February 20, 1945–April 8, 2007) who published numerous classical papers and reviews on tobacco and smoke science during a period of over three decades.

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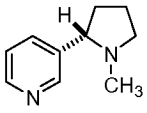
1. Introduction

The increased risk to smokers of contracting many diseases is well known and well documented (US Department of Health and Human Services, 2004). There is also broad agreement that cigarette smoking is addictive and that the tobacco alkaloid (*S*)-(-)-nicotine (**1**) is the addictive agent in mainstream (MS) cigarette smoke (American Psychiatric Association, 2004; Ferrence et al., 2000; Karan et al., 2003; Royal College of Physicians, 2000; Surgeon General, 1988). A smoker's exposure to nicotine is determined by many factors, including the mode of smoking (for example, puff volume and puff frequency) (Baker and Lewis, 2001; Gray et al., 2005; Jarvis et al., 2001; US Department of Health and Human Services October, 2001), the amount of nicotine in the smoke (Callicutt et al., 2006b; Kozlowski et al., 1998), cigarette design features such as filter ventilation and filter efficiency (Norman, 1999), and various physiological factors such as urinary pH, nicotine blood levels and stress (Herning et al., 1983).



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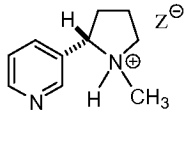
In the last few years, it has been hypothesized that ammonia-forming ingredients[1] added to the tobacco by cigarette manufacturers increase the bioavailability of nicotine to the smoker[2] (Bates et al., 1999; Benowitz and Henningfield, 1994; Gray et al., 2005; Haustein, 2001; Henningfield et al., 2004a; Henningfield et al., 1998; Henningfield et al., 2004b; Hurt and Robertson, 1998; Kessler et al., 1997; Kessler et al., 1996; Pankow, 2001; Pankow et al., 2003a; Pankow et al., 1997; Russell, 1976, 1980; Summerfield, 1999; Wayne et al., 2004, 2006; Willems et al., 2006; World Health Organization, 2007). The extent to which ammonia increases the addictive potential of smoking can, in principle, have serious toxic consequences, i.e., enhanced disease risk to human smokers. Thus, if ammonia increases nicotine bioavailability, considering nicotine's addictive properties, and given the increased risk for disease consequent to smoking, any chemical factor which is thought to increase smoking behavior and nicotine exposure deserves serious attention. Unfortunately, no study has reported the effect of the ammonia on the rate or amount of nicotine reaching the arterial bloodstream or brains of smokers. In the absence of such experimental data, researchers have relied on indirect studies which examine individual "segments" or "subsets" of the tobacco-to-brain sequence.

Nicotine can exist as its free base, non-protonated form **1** and as its protonated forms **2** and **3**. Ammonia and other bases (and acids) in tobacco and in MS smoke can affect the distribution of the three forms of nicotine **1** = **2** = **3** in both tobacco and in MS smoke (Fig. 1). Of the three forms of nicotine, only the semi-volatile, non-protonated, free base form of nicotine (**1**) can volatilize and transfer into MS smoke aerosol. The ammonia-facilitated conversion of **2** and **3** to **1** can, in principle, enhance volatilization of nicotine and its transfer from tobacco to smoke (Fournier et al., 2001; Henningfield et al., 2004b; Seeman, 2005; Seeman et al., 1999; Summerfield, 1999; Willems et al., 2006).



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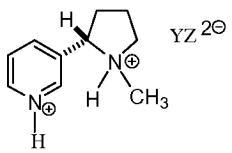

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where Y and Z represent organic carboxylic acid anions found in tobacco and in tobacco smoke

It has been hypothesized that the amount and concentration of non-protonated free base nicotine (**1**) affects the magnitude and rate of nicotine bioavailability to the smoker (Haustein, 2001; Henningfield et al., 2004b; Henningfield and Zeller, 2002; Hurt and Robertson, 1998; Kessler et al., 1997; Pankow, 2001; Pankow et al., 1997, 2003b; Summerfield, 1999; Willems et al., 2006; World Health Organization, 2007). The amount of non-protonated nicotine **1** in any environment equals the product of its fraction in

[1] The term "ingredient" refers to materials *added* to the tobacco by cigarette manufacturers for various purposes, including as flavorants, processing aids, and humectants. "Tobacco blend" refers to the total materials that are encased within the cigarette wrapper including the various tobacco leaf materials, e.g., Burley, Bright and Oriental tobaccos, expanded tobaccos, and reconstituted tobaccos.

[2] A related hypothesis was presented in 1972 in which sugars in tobacco caused an increase in "smoke pH" whereby nicotine's "pharmacological availability is also low because of the progressive increase in acidity during smoking" and the decrease in the fraction of non-protonated nicotine (Elson and Betts, 1972; Elson et al., 1972).

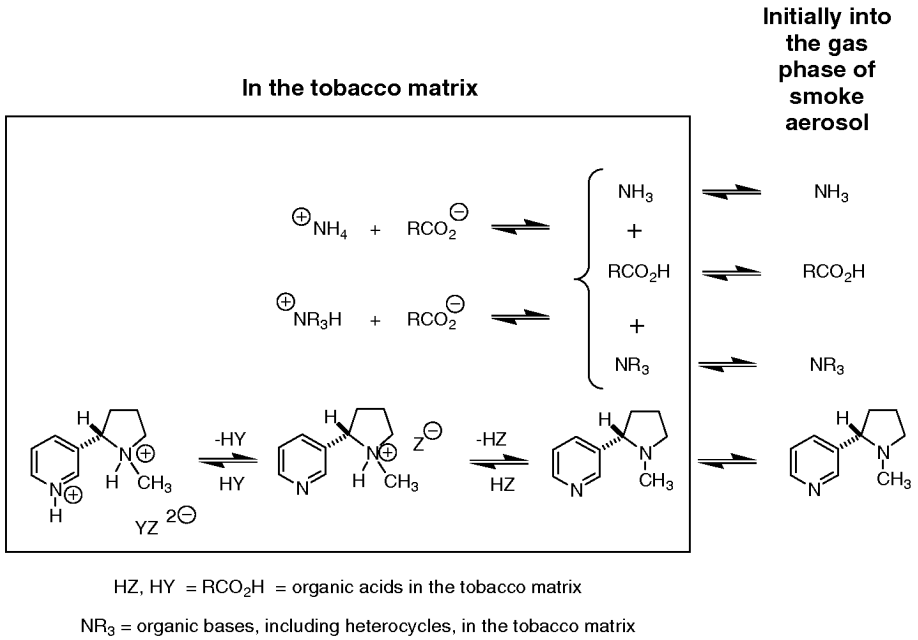


Fig. 1. Illustration of the acid–base interactions within the tobacco matrix and vaporization of the non-charged species to the gas phase of the smoke aerosol. The fractions of the components within the tobacco matrix are not independent but are linked by hydrogen ion via the self-ionization of the acids present and conservation of charge. Some gas phase smoke constituents can condense onto or form particles. Constituents within a particle can participate in acid–base reactions and form salts analogous to those shown within the box above. From Seeman (Seeman, 2007a). This figure can also illustrate the nature of smoke aerosol, if the constituents within the box are considered to be smoke aerosol particles and the constituents outside the box are smoke aerosol gases. Reprinted with permission from Seeman (2007a). Copyright (2007) American Chemical Society.

that environment [sometimes referred to as α_{fb} (Pankow, 2001)] times the total amount of nicotine (**1** + **2** + **3**) (see Eq. (1)). Hence, the total amount of nicotine that transfers into MS smoke and reaches the smoker is of great consequence.

$$[\mathbf{1}] = \alpha_{fb} \cdot \{[\mathbf{1}] + [\mathbf{2}] + [\mathbf{3}]\} \quad (1)$$

where [**1**]–[**3**] = concentrations of **1**–**3**.

This review focuses on a number of interrelated topics, namely (1) the role of ammonia in the hypothesized under-quantification of nicotine in MS smoke during machine-smoking analyses; (2) the absorption of ammonia in the transfer of nicotine from tobacco to smoke; (3) the absorption of ammonia in both MS smoke and environmental tobacco smoke (ETS, also referred to as "second hand smoke" or "passive smoking") in both smokers and non-smokers; (4) the deposition, retention and absorption of mainstream smoke nicotine by smokers and the possible role of ammonia; (5) active and passive transport of nicotine and protonated nicotine salts through the lung–blood interface; (6) the deposition, retention and absorption of nicotine from ETS by anyone in the vicinity of smoking and the possible role of ammonia; and (7) a discussion of risk assessment.

2. Machine-smoking methods for the quantification of MS smoke nicotine and ammonia. Does MS smoke ammonia cause the under-quantification of nicotine in machine-smoking protocols?

MS smoke is a complex heterogeneous, dynamic (Seeman, 2007a) aerosol comprised of particles suspended in a gas (Baker, 1999). Constituents in the particles can evaporate to the gas phase. Gas phase constituents can deposit onto the particles (Ingebrethsen and Lyman, 2002; Pankow, 2001; Seeman, 2007a); see Fig. 1. In order to evaluate the possible role(s) of ammonia on nicotine smoke chemistry and bioavailability, it is important to consider the phase in which these constituents exist (gas phase or in the particles), the form of these constituents (non-protonated or protonated), and their deposition location.

The distinction between the "gas phase" and "gas–vapor phase" of MS smoke is illustrated in Figs. 1 and 2. The "gas phase" of MS smoke refers to the gases in the MS smoke aerosol. The "gas–vapor phase" of MS smoke is, by definition, the material not trapped on the Cambridge filter pad during a machine-smoking. The "gas phase" and the "gas–vapor phases" of MS smoke are not necessarily identical, as material originally trapped on the Cambridge filter pad can possibly evaporate off that pad. In addition, material originally in the gas phase of MS smoke might be trapped onto the Cambridge filter pad.

For the central subjects of this review, it is necessary to establish the reliability of machine-smoking to quantify nicotine in MS smoke. In principle, MS smoke ammonia could increase the amount of nicotine in the gas phase of MS smoke that reached and passed through the Cambridge filter pad (see Fig. 2). If this were to occur, the amount of nicotine trapped on the Cambridge filter pad would decrease, thereby leading to an under-quantification by this machine-smoking procedure of the total amount of nicotine in smoke. In fact, several reports in the literature including a review in this Journal have hypothesized without experimental data that ammonia in MS smoke does lead to the under quantification of nicotine during machine-smoking procedures (Bates et al., 1999; Willems et al., 2006).

A number of studies published from 1973 to 2006 have quantified the amount of nicotine in the gas–vapor phase of MS smoke (Fig. 2). In 1973, Houseman (Houseman, 1973) reported the use of exogenously added ^{14}C-nicotine and reported that 0% nicotine passed through the Cambridge filter pad. In that classic work, Houseman used gas chromatography, ultraviolet spectroscopy, and liquid scintillation counting to make his measurements. In 1999, Stevens and Borgerding (Stevens and Borgerding, 1999) reported the use of exogenously added (*R*,*S*)-nicotine-d_3 (**4**), i.e., a

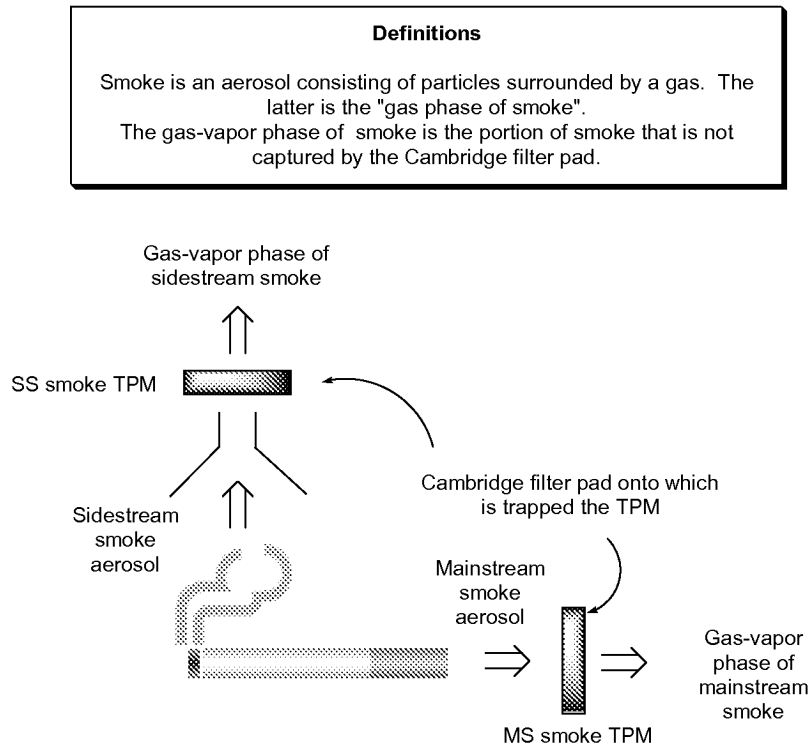
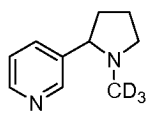


Fig. 2. Schematic illustration of a machine-smoking method to trap and collect the total particulate matter (TPM) on a Cambridge filter pad (CFP) of both mainstream smoke and sidestream smoke. The standard machine-smoking methods used by tobacco companies to report nicotine and tar yields do not include collection of either the TPM or gas–vapor phase of the sidestream smoke. Modified from Callicutt et al. (2006a) and reprinted with permission from *Beitr. Tabakforschung Int.*

deuterated nicotine, to a cigarette and found <1% nicotine in the gas–vapor phase of MS smoke. A major report by the UK Department of Health in 2000 entitled "Determination of the Fate of Nicotine When a Cigarette is Smoked," concluded that, when 50 different brands of commercial cigarettes were smoked by the ISO protocol, there was no evidence for nicotine passing through the Cambridge filter pad (<0.2% of nicotine in the cigarette tobacco) (UK Department of Health, 2001). A similar result was reported by Watson et al. (2004). In 2006, Yu et al. (2006) performed smoking experiments on a commercial 85 mm full flavor filtered cigarette into which was injected (*R,S*)-nicotine-d_4 (**5**). These authors followed four different machine-smoking regimes, the least intense being the FTC/ISO standard and the most intense being the Health-Canada method. They placed a series of traps downstream from the Cambridge filter pad to trap gas–vapor phase constituents. (*R,S*)-Nicotine-d_4 (**5**) was not observed in any of the MS smoke gas–vapor phase traps. In all of the above studies, no specific information is known about the level of ammonia-forming ingredients added to the cigarettes examined. However, as discussed in Section 3, ammonia and ammonia-forming compounds are natural tobacco constituents, and ammonia is found in cigarettes to which no ammonia-forming ingredients are added. Therefore, it is reasonable to expect that ammonia was present in the MS smoke of all of those cigarettes referred to in the immediately above studies.



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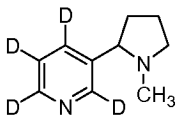

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Only one study has examined the possible effect of ammonia on the quantification of nicotine using machine-smoking methods. In 2006, Callicutt and co-workers published a study in which the content of ammonia-forming ingredients in the tobacco was varied; ammonia in MS smoke also varied, and the amount of nicotine not trapped by the Cambridge filter pad was quantified (Callicutt et al., 2006a). As shown in Table 1, a series of four test cigarettes (T1–T4) and one control cigarette (C, a *Marlboro Lights*® King Size) were examined. The test cigarettes differed from the control cigarette as follows. For US commercial cigarettes manufactured by Philip Morris, when ammonia-forming ingredients are used, they are added to only one type of cigarette blend component, the reconstituted tobacco sheet (and not necessarily to all the reconstituted tobacco sheets in each product) (Callicutt et al., 2006b; Seeman, 2005). Ammonia-forming ingredients were added to the reconstituted tobacco for only C and T1, though in reduced amounts in T1. The soluble ammonia in the tobacco met these design criteria: C > T1 > T2 ≈ T3 ≈ T4. Consistent with these values, MS smoke ammonia was found to be C > T1 > T2 ≈ T3 ≈ T4. For C and T1–T4, ⩾99.89% of the nicotine in MS smoke was quantified by the FTC method, independent of the amount of ammonia in the MS smoke. The Callicutt et al. (2006a) study was performed with sufficient number of cigarettes and replicates that the conclusions were based on robust mean and standard deviation values.

When taken together, the scientific data obtained over more than a 30-year period (Callicutt et al., 2006a; Houseman, 1973; Stevens and Borgerding, 1999; UK Department of Health, 2001; Watson et al., 2004; Yu et al., 2006) establishes that the vast majority (>99%) of the nicotine in MS smoke aerosol, whether in the gases or in the particles, is captured by the Cambridge filter pad and

Table 1
Tobacco and mainstream smoke characteristics of cigarettes with and without ammonia-forming ingredients. Effectiveness of the FTC method to quantify nicotine and a comparison of relative nicotine transfer between the 1998 *Marlboro Lights*® King Size (Control, C) and the test Cigarettes (T1–T4)[a,b]

Tobacco and smoke characteristics	Control and test cigarettes				
	Marlboro Lights® King Size (control, C)	T1	T2	T3	T4
Ammonia-forming ingredients added to reconstituted tobacco in the cigarette blend	Yes	Reduced	No	No	No
Non-ammonia-forming ingredients added to reconstituted tobacco in the cigarette blend	Yes	Yes	Yes	No	No
Ingredients added to the cigarette blend	Yes[c]	Yes[c]	Yes[c]	Yes[c]	No
Soluble ammonia in tobacco (mg/cig)	2.03	1.65	1.06	1.08	1.13
FTC tar (mg/cig)	13.0	12.2	11.8	11.8	10.8
FTC nicotine (mg/cig)	0.83	0.79	0.79	0.77	0.86
MS smoke ammonia (μg/cig)	11.93	10.17	7.07	6.10	6.07
Nicotine collection efficiency of Cambridge filter pad (%)[d]	99.94	99.93	99.94	99.94	99.89
Relative nicotine transfer (RNT) (production run #1)[e]	4.65	4.64	4.58	4.99	4.70
Relative nicotine transfer (Production run #2)[e]	4.76	4.91	4.82	4.81	4.84
Relative nicotine transfer (Production run #2, analysis by second laboratory)[e]	5.06	5.28	5.30	5.25	5.01
Adj. diff. of RNT[f]	Reference	$+0.12^{NS}$	$+0.08^{NS}$	$+0.19^{(+)}$	$+0.02^{NS}$

The *Marlboro Lights*® King Size and T1 have ammonia-forming ingredients in the tobacco blend and have increased MS smoke ammonia relative to T2–T4. Data from Callicutt et al. (2006a) and Callicutt et al. (2006b).

[a] All data from Callicutt et al. (2006b) except for Cambridge filter pad collection efficiency which is from Callicutt et al. (2006a). See these original references for additional experimental data, full experimental details, and complete statistical analyses.

[b] All smoking was performed using the FTC machine-smoking method.

[c] None of these ingredients is ammonia-forming.

[d] Nicotine collection efficiency of Cambridge filter pad equals mass of MS smoke nicotine trapped on the Cambridge filter pad divided by total nicotine in MS smoke nicotine (which equals the former quantity plus the mass of nicotine absorbed onto an XAD-trap placed downstream of the Cambridge filter pad) (Callicutt et al., 2006a). See the original paper for details of number of samples and additional experimental information.

[e] It is necessary to "normalize" or mathematically take into consideration the consequences of different tar yields within the series of cigarettes being compared. Tar or TPM correction is critical (Irwin, 1998; Rustemeier et al., 2002; Seeman et al., 2002; Seeman et al., 2003) in a comparison of nicotine yields between two cigarettes. If one cigarette has a greater overall yield of smoke formation, then an increased nicotine yield may simply reflect more total smoke formation and not a selective effect on nicotine transfer from tobacco to smoke. The parameter "relative nicotine transfer" (RNT, Eq. (i)) was developed by Callicutt et al. (2006b) and Morton et al. (in preparation) and takes into account nicotine's MS smoke yield dependence on tar (Chepiga et al., 2000; Counts et al., 2004, 2005, 2006, 2007; Kozlowski et al., 1998; Swauger et al., 2002). For an earlier derivation of "tar adjusted nicotine transfer" which is similar to RNT, see Irwin, 1998.

$$\text{Relative nicotine transfer (RNT)} = \frac{\text{MS smoke nicotine (mg/cig)/tobacco nicotine (mg/cig)}}{\text{FTC tar (mg/cig)/tobacco weight (mg/cig)}} \quad \text{(i)}$$

[f] For relative nicotine transfer, mean difference from the control over the two production runs and three sets of data. Not statistically significant (NS) $p \geqslant 0.1$. (+) $p < 0.1$.

quantified by the FTC and ISO and more intense Health-Canada machine-smoking regimes.

3. Ammonia in tobacco and in mainstream smoke

Ammonia and ammonia-forming compounds are endogenous constituents of tobacco (Callicutt et al., 2006b; Leffingwell, 1976; Schmeltz and Hoffmann, 1977). While these compounds are found in all tobaccos, Virginia or Bright tobaccos have the lowest concentrations and flue-cured or Burley tobaccos having the highest concentrations. One reported set of measurements found that Bright tobacco and Burley tobacco have ca. 0.02% and 0.16% ammonia, respectively, and 0.065% and 0.2% α-amino nitrogen as ammonia, respectively (Leffingwell, 1999). Typical concentration levels of ammonia in the tobacco blends of commercial U.S. and international cigarettes are ca. 0.02–0.4% (Callicutt et al., 2006b; Counts et al., 2004, 2005, 2006, 2007; Leffingwell, 1999), as measured by the "soluble ammonia method"[3] (Callicutt et al., 2006b; Counts et al., 2004, 2005, 2006, 2007).

A summary listing of ammonia-forming ingredients that may have been used commercially over the years and a range of their possible uses can be found in recent reviews (Dixon et al., 2000; Willems et al., 2006). The Philip Morris USA website (Philip Morris USA, 2007a,b) provides a composite list of ingredients added to its commercial cigarettes. Philip Morris USA lists the "quantity not exceeded" for these ingredients, calculated from the highest level of use in a single brand and expressed as a percentage of the total weight of the tobacco. Two ammonia-forming ingredients are currently found on this Philip Morris USA list (Philip Morris USA, 2007b) [ammonium hydroxide (0.3%, described as a flavor and processing aid) and diammonium phosphate (0.8%, described as a flavor and processing aid)]. These ammonia-forming ingredients are added only to some of the reconstituted tobacco materials in some of the Philip Morris USA cigarette brands (Callicutt et al., 2006b; Seeman, 2005). As processing aids, they release tobacco pectins which bind the tobacco particles together and form the reconstituted sheet (see the following patents and subsequent patents by these inventors: (Hind and Seligman, 1967; Seligman and Hind, 1968)).

Not all the ammonia-forming ingredients added to the tobacco during cigarette manufacture are retained as such in the final commercial cigarettes. The concentration of ammonia-forming ingredients in the final product will be less than the application level for several reasons. First, ammonia will be depleted as a consequence of its application purpose (Seeman, 2005). In addition, ammonia will be lost during application as well as during various manufacturing heating and drying processes (Fisher, 1999; Hind, 1968, 1969Hind, 1968, 1969; Leffingwell, 1999; Norman, 1999). Lastly, ammonia is well known to react with tobacco sugars and other carbohydrates in the Amadori and Maillard reactions (Agyei-Aye et al., 2002; Coleman and Perfetti, 1997; Leffingwell, 1999), in part resulting in the formation of important flavorants (Ledl and Schleicher, 1990), further decreasing ammonia in the final product.

Ammonia is found in both the particles and gas phase of mainstream smoke (Baker, 1999; Callicutt et al., 2006b; Dixon et al., 2000; Schmeltz and Hoffmann, 1977; Seeman, 2007a; Seeman et al., 2004). For any one commercial cigarette, ammonia yields

[3] The "soluble ammonia method" is an aqueous extraction procedure of tobacco that quantifies ammonia and ammonium salts but not precursors of MS smoke ammonia that require hydrolysis, pyrolysis or combustion conditions.

in MS smoke are determined primarily by the smoking conditions (Counts et al., 2005) and also by the blend, as discussed above. In recent tabulations of MS smoke constituent yields, Baker Baker (1999) and Hoffmann and Hoffmann (Hoffmann and Hoffmann, 1998) reported ranges of ammonia yields of 50–130 μg/cig and 10–130 μg/cig using the related ISO- and FTC-machine-smoking methods respectively. Counts et al. (2005) determined the MS smoke yields of ammonia for 48 international Philip Morris brands and the 1R4F Kentucky reference cigarette, when smoked under three different machine-smoking conditions: (in order of increased smoking intensity) ISO, Massachusetts Department of Public Health (MDPH), and Health-Canada (H-C) For these three methods, MS smoke ammonia was 2.5–29 μg/cig, 8.5–58 μg/cig, and 11–90 μg/cig, respectively.

Even in the absence of ammonia-forming ingredients[1] added to the tobacco blend, ammonia is found in MS smoke. For example, the 1998 *Marlboro Lights*® King Size cigarette produced ca. 16.4 μg/cig MS smoke ammonia while similar experimental cigarettes made without added ammonia-forming ingredients to the tobacco blend had MS smoke ammonia yields of ca. 7–9 μg/cig (Callicutt et al., 2006b). Ammonia is formed during smoking from various tobacco-endogenous substrates including amino acids, proteins and inorganic nitrates (Akehurst, 1981; Dixon et al., 2000; Johnson et al., 1973; Leffingwell, 1976; Schmeltz and Hoffmann, 1977). Since more ammonia is found in Burley tobacco than in Bright tobacco as described above (Fenner, 1988; Leffingwell, 1999), it is consistent that there is more ammonia in the MS smoke of single blend Burley tobacco cigarettes or in blended cigarettes than in single blend Bright tobacco cigarettes (Browne, 1990; Counts et al., 2005).

Statistically significant linear relationships have been reported between the yields of MS smoke ammonia and tar for a variety of machine-smoking methods (Arista Laboratories Europe, 2003; Counts et al., 2004, 2005, 2006, 2007; Taylor et al., 2000). This indicates that the amount of ammonia in MS smoke of commercial cigarettes is related primarily to cigarette design features such as filter ventilation that determine overall total smoke formation (Seeman et al., 2002, 2003) and smoking behavior (US Department of Health and Human Services October, 2001). Of course, increasing the amount of ammonia-forming compounds in the tobacco blend generally (Armitage et al., 2004b; Callicutt et al., 2006b; Elson et al., 1972; Saint-Jalm et al., 2000; Sinclair et al., 1998) but not always (Baker et al., 2004) increases the yield of ammonia in MS smoke.

The amount of ammonia in sidestream smoke, the major source of environmental tobacco smoke (ETS) (Guerin et al., 1992; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004), is 50–170 times greater than in MS smoke (Baker, 1999).

4. The contribution of ammonia in cigarette smoke to human smoker toxicity

Ammonia is a pungent, irritating gas. Human exposure to ammonia occurs primarily in the workplace and at much higher doses and for long periods of time than for exposure from tobacco smoke, either during smoking or from ETS. Regulatory agencies have evaluated workplace exposure to ammonia, as referenced below. Unfortunately, no evaluations of ammonia toxicity in the context of smoking are currently available. In order to make an evaluation of the additional potential toxicity of cigarette smoke, which contains thousands of constituents, in the presence of ammonia levels that exceed a non-ammonia-forming ingredient cigarette, the following minimum data are necessary: ammonia exposure during smoking, including its level, site of absorption, and pharmacokinetics of exposure as well as these parameters for the other smoke constituents; and the design and analysis methodologies to assess biological interactions (Gennings et al., 2007a,b).

Regarding the sites of ammonia's absorption, when gaseous ammonia is inhaled, because of its high water solubility, the vast majority is absorbed in the upper respiratory tract (Brautbar et al., 2003). However, most of ammonia in MS smoke is initially in the particles of smoke aerosol. Nonetheless, this ammonia rapidly evaporates from those particles and is thus available for deposition in the mouth and upper respiratory tract from smoke aerosol gases.

It is difficult to quantify human exposure to ammonia during smoking, and therefore conclusive toxicological inferences, i.e., effect impact, are also dangerous. A risk assessment involves both exposure assessment and effect impact. What follows in this section is a discussion of ammonia exposure to smokers. A risk assessment discussion is provided in Section 9.

Ammonia yields in MS smoke using machine-smoking methods are known not to be accurate indicators of human exposure. It is very likely that the yields determined using the most intense machine-smoking method (the Health-Canada method) reflects the upper ranges of human exposure. The Health-Canada method requires the largest puff volumes used in machine-smoking methods (55 mL), with puffs taken every 30 seconds, and with 100% of the ventilation holes blocked. Most smokers do not take puffs every 30 seconds over the course of an entire cigarette; and they do not block 100% of the vent holes (Baker and Lewis, 2001; Harris, 2004; Scherer, 1999).

In the most recent comprehensive study of MS smoke yields of ammonia for commercial cigarettes, Counts et al. (2005) used the Health-Canada protocol and reported yields ranging from 11 to 90 μg/cig with a mean and standard deviation of 39.2 ± 13.8 μg/cig. For the highest yield cigarette, the daily dose of ammonia for a pack-a-day smoker is 1.8 mg (90 μg/cig times 20 cig/d), not 35 mg as calculated by Willems et al., 2006. The value of 1.8 mg/day dose is consistent with the calculation by Sloan and Morie (Sloan and Morie, 1974) reported in 1974 using a protocol standard for the 1970's, over 20 years prior to the development of the Health-Canada protocol. The Health-Canada protocol is the most intense machine-smoking method currently used for regulatory-reporting purposes.

Based on the value of 1 ppm ammonia = 700 μg m^{-3} (Willems et al., 2006), a cigarette yielding 90 μg/cig MS smoke ammonia would have an ammonia concentration of ca. 257 ppm (assuming 10 puffs of 50 mL each). The 257 ppm ammonia concentration applies to the exposure but will be considerably less in the respiratory tract during inhalation of the smoke. An exposure of 257 ppm ammonia is less than the 742 ppm reported by Willems et al., 2006. This of course assumes that all of the ammonia delivered in the puff is available during inhalation, that is, in the lung. In reality, because of the water solubility of ammonia, much of the ammonia in MS smoke will be deposited in the mouth and upper respiratory tract. Furthermore, no one smokes and inhales continuously. Thus, the concentrations of ammonia in smoke in the lung are unknown but clearly exposure of ammonia from smoking is markedly less than the 500 ppm for 30 min that can induce changes in breathing rate (Silverman et al., 1949).

Information on the human toxicity of ammonia is readily available in a number of US Federal and State health and regulatory agency reviews; two highly relevant evaluations/reviews were provided by the California EPA and the US EPA (California Environmental Protection Agency – Office of Environmental Health and Hazard Assessment, 2007; US Environmental Protection Agency, 1995). The data and conclusions utilized in these agency reviews are quite similar. In the only chronic human exposure study to ammonia, in 52 workers and 31 controls in a soda ash plant,

Holness et al. (1989) evaluated pulmonary function, respiratory symptoms, and eye and skin effects. The subjects were exposed during work hours on-average for more than 12 years to approximately 9 ppm of ammonia whereas controls were exposed to approximately <0.5 ppm. No difference in any end points over the work shift was reported between the two groups. In 1995, Cal EPA (California Environmental Protection Agency – Office of Environmental Health and Hazard Assessment, 2007) characterized the Holness et al. (1989) study as "the only chronic study [of ammonia] available... published in a respected, peer-reviewed journal." In these cases, the basis for the human health effects are the local effects of ammonia, i.e., the irritating effects of ammonia on the respiratory tract as opposed to the systemically available ammonia.

In another study, Ferguson et al. (1977) evaluated healthy human volunteers who were exposed to 25, 50, or 100 ppm of ammonia, five days per week, and two or four or six hours each day for six weeks. They examined pulmonary function and irritation of the eyes and respiratory tract. The authors reported transient irritation of the nose and throat at 50 or 100 ppm. Adaptation to eye, nose and throat irritation was observed following 2–3 week of exposure. They concluded that exposure to 100 ppm of ammonia with transient elevations to 200 ppm of ammonia were easily tolerated by the subjects and had no observed effects on their general health.

From the data of Counts et al. (2004) as discussed above, a mouth level exposure of ammonia of ca. 25–290 ppm during smoking can be estimated. Even smoking two packs per day at what is considered to be the near upper limit of human smoking behavior leads to a total time of exposure of ca. 120 min per day. At the highest levels of estimated ammonia exposure due to human smoking, the most relevant research in the literature (Ferguson et al., 1977; Holness et al., 1989) would suggest little or no difference in any of the measured biological endpoints. Regarding the possibility of toxicological interaction due to the thousands of other smoke constituents, any extrapolation to human smoking must be done with extreme caution. No toxicological data currently exists to allow even a first-pass estimation of the contribution of ammonia in tobacco smoke to that smoke's toxicity.

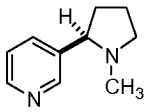
5. Transfer of nicotine from tobacco to smoke aerosol

5.1. Thermal studies as models for cigarette smoke formation

In tobacco, nicotine is most likely present as its nicotine carboxylic acid salts **2** and **3** (Seeman et al., 1999). Nicotine salts **2** and **3** in tobacco must be first converted to non-protonated nicotine (**1**) prior to nicotine's volatilization and transfer to the gas phase. *Upon being heated in the presence of oxygen, do* **2** and **3** *thermally decompose, destroying the nicotine ring system, or do they form non-protonated nicotine* (***1***) which volatilizes (Eq. (2))? Does ammonia enhance the transfer of nicotine carboxylic acid salts to nicotine in the smoke aerosol? A number of papers have examined these very questions.

For many years, thermal studies have been conducted as model for smoke formation (Baker and Bishop, 2004; Stotesbury et al., 1999, 2000; Wooten et al., 2004) and to understand the transfer of nicotine from tobacco to smoke. When nicotine was injected into a preheated pyrolysis chamber in an inert atmosphere, it begins to decompose at temperatures greater than ca. 600 °C (Jarboe and Rosene, 1961; Kaburaki et al., 1970; Schmeltz et al., 1972, 1979; Woodward et al., 1944). When pyrolyzed in an atmosphere of air, nicotine decomposes at ca. 300 °C (Kobashi et al., 1963; Kobashi and Sakaguchi, 1960). However, during the puffing or smoldering of a cigarette, nicotine does not reach those high temperatures. And the region directly behind the cigarette coal is oxygen deficient (Baker, 1999). Nicotine, when heated from room temperature in a flow of gases, is transferred to the gas phase at temperatures below 200 °C (Fournier et al., 2001; Riggs and Perfetti, 2001; Seeman et al., 1999). Experimental evidence for this conclusion is now presented.

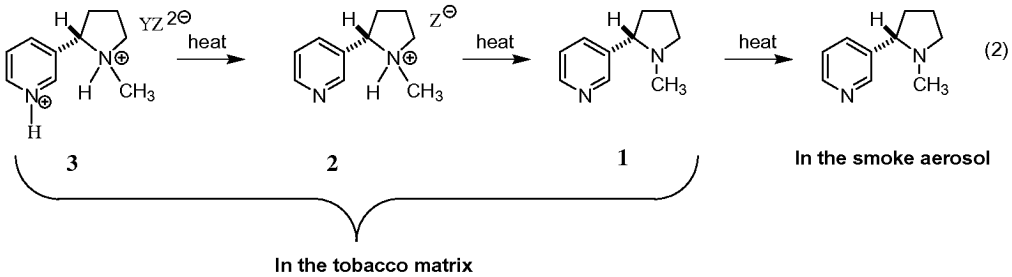
Thermogravimetric (TG) analysis studies have demonstrated that nicotine is transferred to the gas phase at temperatures far below nicotine's thermal decomposition threshold (Fournier et al., 2001; Riggs and Perfetti, 2001; Seeman et al., 1999). When nicotine and protonated nicotines are heated in a flow of gases, nicotine is transferred to the aerosol in near quantitative yield without racemization to its enantiomer **6**, i.e., (*R*)-(+)-nicotine (Fournier et al., 2001). Three different types of chemical reactions have been proposed to explain the relative ease in which nicotine carboxylic acid salts **2** and **3** as found in tobacco are converted to non-protonated nicotine (**1**): acid–base dissociation, thermal decomposition of the carboxylic acid anion, and disproportionation (Seeman, 2005; Seeman et al., 1999). Support for these thermal models comes from a complex smoking study by Baker (1987) who reported that the concentration of nicotine behind the coal in a cigarette during continuous draw is extremely low in the temperature ranges above ca. 300 °C (Baker, 1987).



6

(*R*)-(+)-nicotine

In summary, the nicotine ring system is relatively thermally stable; non-protonated nicotine (**1**) and nicotine carboxylic acid salts (**2** and **3**) as found in tobacco can be converted to **1** at temperatures below those required to thermally decompose the nicotine ring system; and nicotine volatilizes to the gas phase prior to substantial thermal or oxidative decomposition. These experiments provide evidence that nicotine transfers from tobacco to smoke during smoking without the need for exogenous bases such as ammonia to be added to the tobacco.



5.2. On the postulated role of ammonia on the transfer of nicotine from tobacco to mainstream smoke

The most direct way to evaluate the effect of ingredients on the transfer of nicotine from tobacco to MS smoke is to compare a series of cigarettes having various levels of the additives(s) with a control cigarette have zero additive(s). It is necessary to know both the amount of nicotine in the tobacco and the yield of nicotine in MS smoke. It is also necessary to "normalize" for overall smoke formation, as discussed in the literature (Carmines, 2002; Rickert et al., 2007; Rustemeier et al., 2002; Seeman et al., 2003) and in footnote e of Table 1. Willems et al. (2006) have cited a number of studies in tobacco company documents available on the internet, but the experiments reported in the original documents unfortunately do not contain the requisite data, lack the appropriate controls, and are not amenable to statistical analysis.

Callicutt et al. (2006b) have published the only study that contains all of the required experimental data to compare nicotine transfer from tobacco to mainstream smoke in commercial-type cigarettes as a function of ammonia-forming ingredients in the tobacco and ammonia in the MS smoke. A control cigarette (C, a *Marlboro Lights*® King Size cigarette) and four test cigarettes (T1–T4, see Table 1) were examined. The test cigarettes differed from the control cigarette by having a stepwise reduction in ammonia-forming ingredients that were added to the tobaccos and also by a variation in all the other non-ammonia ingredients added to the tobacco blend. The levels in ammonia-forming ingredients in this series of cigarettes range from the levels found in the *Marlboro Lights*® King Size cigarette to zero additives. Thus, without exceeding commercial-use levels for this cigarette, this is the widest range in additives possible (zero to full application level). Exceeding commercial use levels of additives can, in principle, alter the physical and chemical properties of the cigarette.

All smoke values were obtained using the FTC machine-smoking protocol. For cigarettes having ammonia-forming ingredients added to the tobacco blend (C and T1), statistically significant increases in MS smoke ammonia were observed. As detailed in Table 1 (Footnote e), the parameter relative nicotine transfer (RNT) was derived to quantify the transfer of nicotine from tobacco to smoke (Callicutt et al., 2006b; Irwin, 1998; Morton et al., in preparation). For the 15 values of nicotine transfer as quantified by RNT, there is no statistically significant ($p < 0.05$) evidence that nicotine transfer from tobacco to smoke varied by cigarette type. There is also no statistically significant *trend* in nicotine transfer over the five cigarette types, C and T1–T4. If the ammonia-manipulation hypothesis were correct, RNT for the control cigarette would have been greater than that of the other four cigarettes; and RNT for T1 would have been greater than T2–T4. No statistically significant relationship was found between RNT and "tobacco pH" or between RNT and "smoke pH".

The experimental data demonstrate that neither ammonia-forming compounds added to the tobacco nor ammonia in the mainstream smoke increased the nicotine transfer in the *Marlboro Lights*® King Size cigarette. In fact, there was a slight increase in nicotine transfer for one of the test cigarettes (T3) that had less ammonia-forming ingredients in the tobacco and less ammonia in MS smoke. These smoking studies are consistent with the thermal properties of nicotine and its carboxylic acid salts as found in tobacco and the temperature profiles in the burning and puffing cigarette (see discussion in Section 5.1).

6. Effect of ammonia on absorption of nicotine by smokers

6.1. Effect of ammonia on oral absorption of nicotine from MS smoke

Several studies indicate that nicotine absorption in the buccal cavity can be affected by the pH of the medium, in instances when the buffering capacity of the buccal cavity is exceeded. This can occur with moist snuff (Fant et al., 1999; Surgeon General, 1988; Tomar and Henningfield, 1997) or by pretreatment of the mouth with basic materials (Armitage and Turner, 1970; Burch et al., 1993; Henningfield et al., 1990). In the study by Burch et al. (1993), it is possible that the high basicity of the aerosol (pH of aqueous solutions of the aerosol source were 11) itself might injure the buccal cavity membranes, leading to increased nicotine absorption.

Nicotine absorption in the buccal and upper airways is far less efficient in terms of the rate and quantity of nicotine delivery eventually to the brain than nicotine deposition in the lower lung (Benowitz et al., 1988; Bergström et al., 1995; Dixon et al., 2000; Hukkanen et al., 2005; Karan et al., 2003; Lunell et al., 1996, 2000; Molander et al., 1996; Schneider et al., 1996; Schuh et al., 1997; Seeman, 2007a). This is because absorption in the mouth/upper airway involves the venous return system which involves (a) hepatic metabolism and rapid nicotine clearance; and (b) longer circulation time before the nicotine reaches the brain. Nicotine absorption in the lower lung leads to more copious blood supply in the gas exchange area of the alveoli and direct absorption straight into pulmonary circulation. *Thus, to the extent that ammonia or other bases increase the deposition, retention and absorption of nicotine in the buccal cavity and upper respiratory tract during human smoking at the expense of nicotine deposition and absorption in the lower respiratory tract, for the same amount of nicotine exposure, the total rate and amount of nicotine to the bloodstream and to the CNS will decrease.* For more details and comparisons of these various routes of absorption and the nicotine pharmacokinetics much of which is now decades old, see the work of Russell, Benowitz, Lunell and others (Benowitz, 1998a; Karan et al., 2003; Lunell et al., 1996; Russell and Feyerabend, 1978; Surgeon General, 1988).

6.2. Effect of ammonia on pulmonary absorption of nicotine from MS smoke. Active and passive transport

The following hypothesis has been made in the literature (Bates et al., 1999; Gray et al., 2005; Haustein, 2001, 2004a; Henningfield et al., 2004b; Pankow, 2001; Willems et al., 2006): that ammonia-forming ingredients in the tobacco or other cigarette modifications increase the percent of uncharged, non-protonated nicotine; that a greater fraction of non-protonated nicotine at the lung–blood interface leads to an increased amount and rate of nicotine transferring into the arterial bloodstream; and that this increases nicotine's amount and rate of transfer to the brain of smokers, thereby enhancing nicotine's dependence producing properties.

Greater than 99% of the nicotine in MS smoke aerosol is in the particles as the smoke aerosol exits the cigarette (Lewis et al., 1994, 1995; Lipowicz and Piade, 2004; Pankow, 2001). For cigarette smokers who inhale, the MS smoke aerosol is transferred from the buccal cavity and through the trachea and bronchi into the deep lung (Baker and Dixon, 2006). Many studies over several decades have consistently found that >90% and perhaps nearly all the nicotine in MS smoke that is inhaled by smokers is retained (Armitage et al., 2004a,b; Baker and Dixon, 2006). Thus, given nicotine's nearly quantitative retention during inhalation, it is unlikely that any ingredient can increase the percent nicotine retention. (In Section 5, it has been demonstrated that neither ammonia-forming ingredients in the tobacco nor ammonia in the MS smoke increases the amount of transfer of nicotine from tobacco to the smoke.) We now discuss whether ammonia can enhance the rate of nicotine transfer to the arterial bloodstream and brains of smokers.

Fig. 3 illustrates the two predominant mechanisms by which nicotine in smoke can deposit onto and be absorbed into the lung–blood interface. Mechanism A involves the direct deposition of nicotine-containing particles followed by diffusion of nicotine

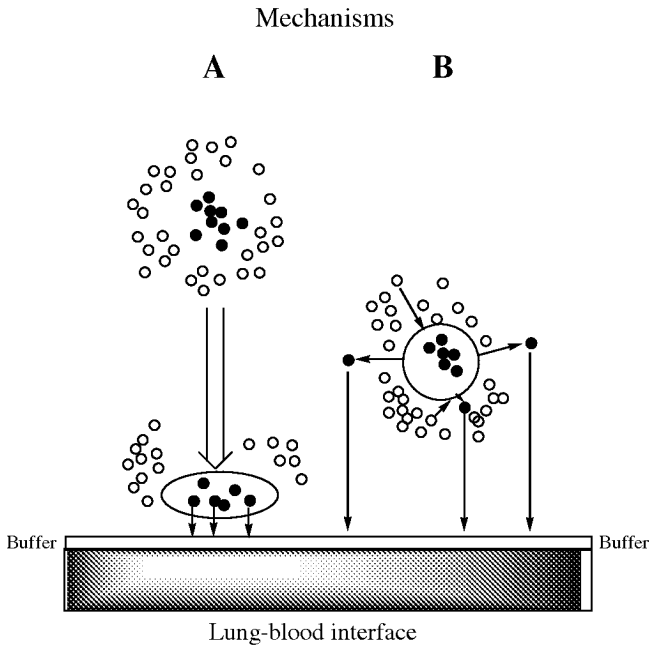


Fig. 3. This graphic illustrates the two predominant mechanisms by which nicotine deposits and is absorbed in the lung. Large circles and the oval represent particles in MS smoke aerosol. Dark circles represent nicotine. Small open circles represent gas-phase constituents. Greater than 99% of the nicotine in MS smoke is in the particles. (A) Particles containing nicotine deposit onto lung–blood interface, and nicotine diffuses through the particle and into the buffer and then into the lung–blood interface. (B) Nicotine evaporates from a particle. Then the resultant gas-phase nicotine molecule diffuses through the lung, deposits onto the buffer, and then transfers into the lung–blood interface. Modified from Seeman (2007a) and Pankow (2001). Reprinted with permission. Copyright 2007 and 2001, respectively, American Chemical Society.

into and through the buffer and into and through the lung–blood interface into the arterial blood. Mechanism B involves the evaporation of nicotine from particles followed by deposition of gas phase nicotine onto the lung–blood interface. These two mechanisms will be discussed in turn.

6.2.1. Mechanism A: direct deposition of nicotine-containing particles followed by transport of nicotine through the lung–blood interface. Active and passive transfer

Acids and bases, including ammonia, in tobacco smoke particles are not likely to alter the pH at the luminal side of the airways (Hukkanen et al., 2005; Seeman, 2007a; Willems et al., 2006). This is because of the high buffering capacity in the lining fluid of the human lung, the small amount of nicotine per puff (<0.3 mg/puff) and the huge surface area of the lung upon which MS smoke particles deposit. Furthermore, for smoke particles that reach the lower respiratory tract, it is likely that the ammonia initially in the gas phase of MS smoke and most of the ammonia initially in the MS smoke particles will have already evaporated and been absorbed in the buccal cavity and the upper respiratory passages.

Smoke particles containing nicotine can directly deposit onto the lung–blood interface (Fig. 3A). We now discuss the two fundamentally different transport mechanisms by which nicotine can pass from the lung–blood interface into the blood and then on to the brain of smokers: passive transport and active transport. First, we discuss passive transport.

There has been a general consensus in the tobacco literature over the past 20 years that, regarding nicotine's transport through biological membranes, membrane permeability is controlled by the charge characteristics of the substrate (Benowitz, 1988, 1998b; Benowitz et al., 1990; Hukkanen et al., 2005; Karan et al., 2003; Surgeon General, 1988; Willems et al., 2006). This theory simply states that small uncharged molecules have higher lipid partitioning properties thereby permeating biological membranes, which are lipid bi-layers, with greater ease than their charged forms. Once nicotine reaches the buffered liquid phase at the lung–blood interface, the relative concentration of the forms of nicotine (**1**–**3**) will be determined by the pH of that buffer (pH 6.9) (Joseph et al., 2002). See Fig. 4. It is generally believed that only the non-protonated, uncharged form of nicotine (**1**) will transfer through the lung–blood interface.

A recent review stated, "Considering the pH-value of the lining fluid (pH of 7.6), about half of the free-base nicotine will be protonated again, leaving the remaining non-protonated nicotine available for rapid absorption" (Willems et al., 2006). This statement assumes that the other half of the nicotine, the half that is initially in the protonated form **2**, is unavailable for rapid absorption. In fact, the equilibrium **1** = **2** shown on the left side of Fig. 4 is rapid

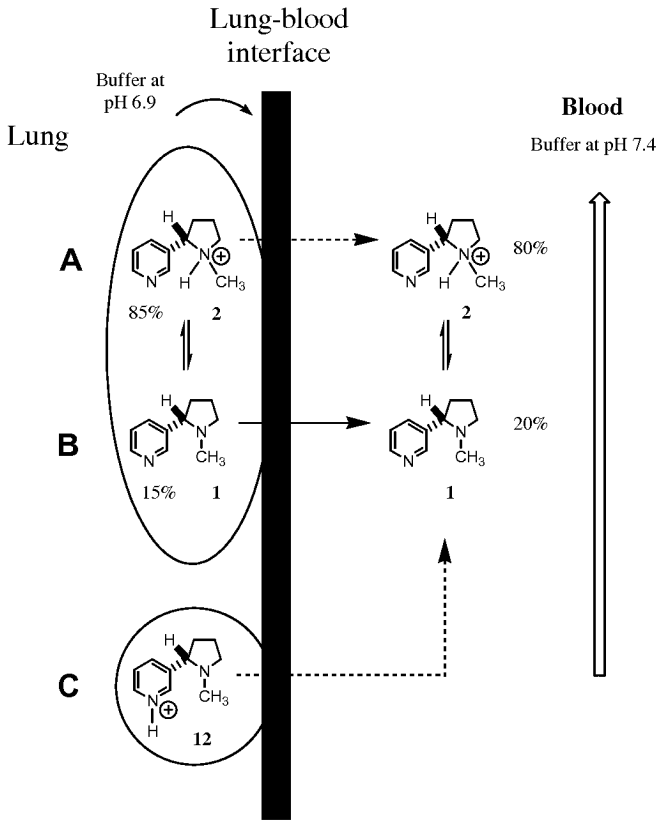


Fig. 4. Graphical representation of equilibration **1** = **2** at the lung–blood interface and in the blood and passive and active transfer of nicotine through the lung–blood interface. For simplicity, diprotonated nicotine **3** is not shown because at these pH values, its concentration is very low. The double arrows signify the extremely rapid rate of proton transfer (acid–base reactions) compared to the rate of transfer of nicotine across the lung–blood interface. (B) The solid single arrow across the lung–blood interface (B) signifies both the transfer of the well-accepted passive transfer route of the non-protonated, non-ionized species **1** into the bloodstream as well as active transport of nonprotonated nicotine. As non-protonated nicotine (**1**) transfers into the interface, the equilibrium **1** = **2** is rapidly reestablished because of the rapidity of proton transfers. Once **1** reaches the bloodstream, the buffering of the bloodstream rapidly reestablishes the equilibrium distribution of **1** = **2**. The continuous reestablishment of the **1** = **2** equilibrium permits all of the nicotine to pass through the lung–blood interface. Of course, other pathways are possible. For example, some of the nicotine can metabolize in the lung. One of the dashed arrows (A) across the lung–blood interface illustrates the potential active transfer of the monoprotonated form of nicotine **2.** Results published by Nair et al., 1997 suggests that this route may be possible. See text regarding the active transport of **12** through the lung–blood interface (C). Modified from Seeman (2007a). Reprinted with permission. Copyright (2007) American Chemical Society.

because acid–base proton transfer reactions under these conditions are extremely rapid. Non-protonated nicotine (**1**) and protonated nicotine (**2**) interconvert much faster than nicotine is transferred through the lung–blood interface (Brewer et al., 2004; Rose et al., 1999). As non-protonated nicotine **1** is absorbed into and transfers through the lung–blood interface, there is a momentary time period ($\ll$1 ms) during which there is a net decrease in the fraction of non-protonated nicotine on the luminal side of the lung–blood interface. However, the **1** = **2** equilibrium is rapidly restored. Whatever the rate of transfer of non-protonated nicotine into and through the lung–blood interfaces, eventually all the nicotine will transfer (of course, some of the nicotine may be metabolized and thus converted to other substances (Gorrod and Jacob, 1999)). This is a broadly generalizable characteristic of passive absorption across lipid membranes. The slow drain (into the lung–blood interface) from the pool of nicotine undergoing fast equilibration (acid–base interconversions of nicotine) shown in Fig. 4 is generalized by Eq. (3). For a full discussion of the kinetics of a system generalized by Eq. (3), see the review by Seeman (1983).

$$A_1 \overset{\text{fast}}{\rightleftharpoons} A_2 \overset{\text{slow}}{\rightarrow} A_3 \qquad (3)$$

Thus, regardless of the fraction of the nicotine that is protonated as it reaches the luminal side of the lung–blood interface, all of the nicotine will be available for transfer due to rapid re-equilibration of the two forms of nicotine, as shown in Eq. 3 and Fig. 4 (Seeman, 1983, 2007a). Accordingly, it does not matter whether nicotine arrives at the lung–blood interface entirely as **1**, or entirely as **2**, or even some combination of **1**–**3**. And similarly, the presence or absence of ammonia in the mixture will not matter either; any ammonia or ammonium salt will also react with the buffer of the lung–blood interface.

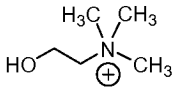
In addition, ammonia is highly water soluble gas. As pointed out by Willems et al., 2006, most if not all of the ammonia initially present in MS smoke will deposit in the buccal cavity and upper respiratory tract and will therefore not be present in the lower lung to affect these processes in that location. Therefore, regarding passive transport and Mechanism A (Fig. 3), ammonia initially in the MS smoke aerosol will have little or no influence on the amount and rate of transfer of nicotine transferred from the lung–blood interface into the blood.

We now discuss the second mechanism, active transport of nicotine through the lung–blood interface (Fig. 4). In principle, all the forms of nicotine (**1**, **2**, **3**, and **12**) can be substrates for active transport through the lung–blood interface. It has been experimentally demonstrated that the transport of nicotine can also occur via active transport systems in brain, kidney, placenta, enterocytes, and hepatocytes (Allen and Lockman, 2003; Allen et al., 2003; Fukada et al., 2002; Spector and Goldberg, 1982). The lung plays a major role both for nicotine's systemic absorption as well as being a target organ for many of smoking's adverse health effects such as lung cancer and chronic obstructive pulmonary disease (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004). In a recent review, von Wichert and Seifart (2005) describe absorptive mechanisms for a wide range of inhaled substances such as water, electrolytes, drugs, lipids, proteins, and peptides. These authors explain that the classical view of passive transport alone is inadequate, and that both active and passive mechanisms must be considered.

Indeed, Waddell and Marlowe (1976) reported more than three decades ago that radioactive nicotine, when administered iv, sc, ip or by inhalation in mice and rabbits, accumulated to various degrees in a number of tissues. The bronchi and the kidneys were found to be one of the major sites of accumulation of radioactivity. At the time, these authors stated that they could not distinguish between metabolism and active transport. Spector and Goldberg (1982) reported that, in an *in vitro* study of the choroid plexus from rabbits, the transport of ^{14}C-nicotine demonstrated properties that included a large (>90%) active transport component. These authors further stated that "the very high levels of nicotine in the brain cannot be explained by passive processes" (Spector and Goldberg, 1982).

Nair et al. (1997) provided experimental evidence that suggests that protonated nicotine can be transported through the lung–blood interface in humans (Fig. 4A and possibly even Fig. 4C). These researchers evaluated the permeation kinetics of nicotine in a number of porcine tissues at various pHs (2.0–8.8). Nicotine permeation kinetics for transdermal and transoralmucosal follow the theory (discussed above) that an increase in the percent of charged species (**2** and **3**) will decrease permeation kinetics. The only deviation in this study to the above relationship was observed in nasal mucosa tissue, in that permeation kinetics was independent of the pH of the buffered nicotine solution, i.e., independent of the relative concentration of the form(s) of nicotine at the membrane interface. Nair et al. suggested that the pH insensitivity observed for the nasal mucosa could occur by mechanisms other than lipid partitioning such as active transport. The nasal mucosa contain respiratory epithelium (Crafts, 1979) similar to that found in the lung. Nair et al. also point out that the distinctions observed in nicotine kinetics in the porcine model are generalizable to humans as well. That is, passive transport occurs in certain areas, for example upper respiratory buccal and dermal in both porcine and humans; and active and passive transport may both occur in respiratory epithelium in human and nasal mucosa in porcine.

Subsequent experimental work by Allen and Lockman (2003) and Allen et al. (2003) have identified the choline (**7**) transporter as a mode for actively transporting nicotine into the brain as well as other tissues or organs.



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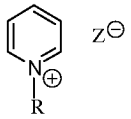
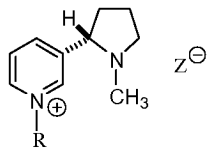
Choline is a salt having a low molecular weight organic cation and an anion determined by the buffer system. Importantly, choline, an essential substrate for membrane formation and acetylcholine synthesis, has minimal passive transport properties (Allen et al., 2003). The choline active transporter is ubiquitous in the body (Allen et al., 2003; Spector and Goldberg, 1982). Nicotine and a number of quaternary pyridinium salts **8** and nicotinium salts **9** are substrates for the choline active transporter; the characteristics of this transporter and these substrates have been described and some data is listed along with the structures below (Allen and Lockman, 2003; Allen et al., 2003; Spector and Goldberg, 1982).



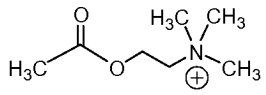
8a, R = CH_3; K_i = 4000 mM
8b, R = n-C_8H_{17}; K_i = 32 mM



9a, R = CH_3; K_i = 2000 mM
9b, R = n-C_4H_{17}; K_i = 777 mM
9c, R = n-C_8H_{17}; K_i = 49 mM
9d, R = n-$C_{10}H_{21}$; K_i = 27 mM

We now suggest the possibility that protonated nicotine(s) *may* be substrates for the choline active transporter. That nicotine can

co-opt a physiological system is based on the classical analogy of the nicotinic–cholinergic receptors throughout the body including within the CNS. Protonated nicotine (**2**) might also be a substrate for the choline active transporter (see Fig. 4A) in that the charged species choline (**7**) and also quaternary salts **8** and **9** are actively transported across membranes. Acetylchoine (**10**) has its own transporter system (Varoqui and Erickson, 1996).



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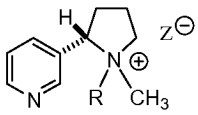
There are at least two caveats regarding the suggestion that active transport may occur for protonated nicotine. (1) In aqueous conditions, nicotine's pyrrolidine ring nitrogen is more basic than its pyridine ring nitrogen. Hence, nicotine in aqueous solutions of pH ca. 6–8 is primarily in its monoprotonated form **2** and not the monoprotonated form **12**. Allen and Lockman (2003) and Allen et al. (2003) reported only the pyridinium quaternary salts **8** and **9** were substrates for the choline active transporter; they did not report any results for nicotinium salts quaternized on the pyrrolidine ring although these types of compounds are known, such as **13** (Seeman and Whidby, 1976). (2) Allen et al. (2003) also reported that the inhibition of ^{3}H-choline transport by the four quaternary nicotine salts **9a–9d**. The analogues having the highest affinity for the transporter had the largest pendent alkyl substituents, suggesting that a lipophilic substituent increases binding to the transporter. In principle, it is possible that protonated nicotine analogues **2** or **12** could be present at the active site and be substrates for active transport (Fig. 4C). Of course, **12** is less lipophilic than **9d**. These ideas provide ample territory for further experimental investigations. Nonetheless, suffice it to say that any active transport of protonated nicotine(s) would further minimize the possible role of ammonia in increasing the amount or rate of nicotine transfer to the blood. Unfortunately, no quantitative data on the relative rates of transport of the different forms of nicotine (**1–3**) in the human lung exist.



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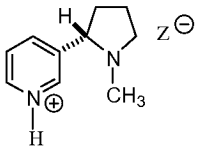

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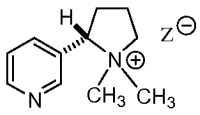

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There is additional experimental evidence that the lung–blood interface is not a simple passive membrane system. In addition to the buffering differences in the lung (pH 6.9) and the blood (7.4), there are also important cellular anatomical and functional differences that further demonstrate that the lung is much more than a passive membrane system. Systems that require transport uphill from a natural gradient require active transport. Thus, Joseph et al. (2002) have shown that the alveolar epithelium of the lung exhibit anatomical and functional polarity which differentially effects the apical (that portion exposed to air) from the basolateral (that region in contact with the blood) with respect to proton transport and gradient maintenance. The anatomy of the kidney also shows this anatomical dichotomy, though the flow of materials is in the opposite direction due to the kidney's excretory function.

In summary, there are two mechanisms for the transport of nicotine through the lung–blood interface: passive and active. The distribution and transport of all the forms of nicotine via both active and passive transport mechanisms have not been considered in the reports proposing nicotine manipulation by ammonia (see, for example: Bates et al., 1999; Haustein, 2001; Henningfield et al., 2004b; Kessler, 1994; Kessler et al., 1997; Pankow, 2001; Willems et al., 2006). Regardless of the proportion of each transport pathway within the lungs of the smoker and its specific substrate, rapid re-equilibration of **1** = **2** will be maintained in the buffer. This will further mitigate against any direct effect of ammonia or other acids and bases in tobacco smoke on enhancing the bioavailability of nicotine in the smoker.

Any delay in the transport of nicotine across the lung–blood interface – whether by active or passive transport – would also decrease the likelihood of ammonia or any acid–base system within MS smoke affecting the rate of transfer. Rose et al. (1999) provided evidence and concluded that nicotine is retained by the lung "on the order of 1–2 min[utes]" (Rose et al., 1999) in contrast with a recent (2003) estimate (Karan et al., 2003) of 10–19 s for the time on nicotine transfer from the lung to the brain of smokers. More recently, Brewer et al. (2004) provided additional support for a residence time of nicotine in the lung–blood interface of the order of a minute or longer. The operation of these transport mechanisms to nicotine and MS smoke is far more complex than a simple inspection would imply.

6.2.2. Mechanism B: evaporation of nicotine from particles followed by deposition of gas phase nicotine onto the lung–blood interface

The first mechanism that we considered for nicotine deposition was direct deposition of nicotine-containing particles onto the lung–blood interface (Fig. 3A and Section 6.2.1). The second mechanism for nicotine deposition involves the evaporation of nicotine from tobacco smoke particles followed by deposition of gas phase nicotine onto the lung–blood interface (Fig. 3B). As shown in Fig. 1, only non-protonated nicotine (**1**) is sufficiently volatile to evaporate from the smoke aerosol particles. The protonated nicotine salts **2** and **3** are non-volatile. In principle, any factor which selectively increases the concentration of **1** in the smoke particles will increase the rate of evaporation of nicotine from those particles. As ammonia is a stronger base than is nicotine, holding everything else constant, increasing the concentration of ammonia in MS smoke particles will, in principle, increase the concentration of **1** in those particles and therefore increase the rate of evaporation of nicotine from those particles.

The evaporation of nicotine in smoke particles can be enhanced by ammonia *only if that ammonia is in those particles*. Ammonia is a gas at room temperature (bp −33 °C). In contrast, nicotine is a high boiling point liquid (bp 247 °C). Therefore, and rather unsurprisingly, ammonia evaporates from smoke particles faster than does nicotine (Ingebrethsen et al., 2001; Seeman, 2007a; Seeman et al., 2004). Thus, if ammonia is to enhance the evaporation of nicotine from tobacco smoke aerosol particles, it must be early in the lifetime of the particles before the ammonia has completely evaporated from the particles. Ammonia-enhanced evaporation of nicotine would occur, if at all, in the mouth and upper airways of the smokers' lungs. The rate and amount of nicotine transfer to the bloodstream from the mouth and upper airways is much less than from the lower lungs, as discussed more fully with references in Section 6.1. Hence, ammonia-facilitated evaporation in the mouth and upper airways would effectively decrease nicotine's total bioavailability to the smoker.

We now examine experiments reported in the literature (Liang and Pankow, 1996; Pankow et al., 1997) that have been used to support the hypothesis that ammonia increases the rate of evaporation of nicotine from smoke particles (Haustein, 2001; Pankow, 2001; Stephens, 2007; Wayne et al., 2006; Willems et al., 2006). The experimental data (Liang and Pankow, 1996; Pankow et al., 1997) fail to support a ammonia-enhancement of nicotine evaporation from MS smoke aerosol particles in the lungs for several reasons. (1) These experiments were performed on trapped, aged

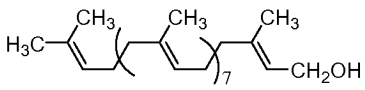
mainstream smoke particulate matter and also on trapped, aged environmental tobacco smoke particulate matter, not on fresh, dynamic smoke. (2) The equations upon which the experimental data was analyzed require a system that is at equilibrium (Liang and Pankow, 1996; Rounds et al., 1993). In contrast, MS smoke aerosol is a dynamic, rapidly changing system. (3) The underlying theory (Rounds et al., 1993) used in the data analysis is inconsistent with the experimental design (Pankow et al., 1997). That is, the theory and experimental design were developed for non-reacting desorbing systems (Rounds et al., 1993). In contrast, the experiments were intentionally performed on reactive systems, namely ammonia reacting with protonated nicotines **2** and **3** to form ammonium salts and non-protonated nicotine (Pankow et al., 1997). (**4**) The experimental data that showed the major effect (a 129-fold increase in nicotine's volatility under the ammonia-fortified conditions) was from the trapped, aged ETS sample even though, in the publication, the sample was misidentified as a MS smoke sample (compare Tables 1 and 3 from the original reference (Pankow et al., 1997)). Essentially no ammonia enhancement in nicotine's volatility was observed with the trapped, aged MS smoke sample. Additional reexamination and reevaluation of these experiments and the literature conclusions (Pankow et al., 1997) are reported in detail in a recent study (Seeman, 2007a) and in the Supplementary Information of that study (Seeman, 2007b). Suffice it to say that the experimental data on both fresh mainstream smoke aerosols as well as on trapped, aged mainstream smoke particulate matter are consistent with a non-ammonia effect on the evaporation of nicotine from MS smoke aerosol particles.

6.2.3. Experimental studies on nicotine retention during human smoking as a function of ammonia

In 2004, Armitage et al. (2004b) published the *only* study in which the effect of added ammonia-forming ingredients to tobacco (and consequently, increased ammonia in smoke) on nicotine retention and systemic absorption by human smokers were evaluated. These researchers only measured nicotine venous blood levels following smoking. As shown in Table 2, they prepared two test cigarettes (T5 and T6), each of which had ammonia-forming ingredients incorporated into the tobacco blend. Consequently, both T5 and T6 had greater amounts of MS smoke ammonia than the control cigarette K. In the 2-s mouth-hold only experiment, i.e., no inhalation, nicotine retention was T5 (64 ± 11%) > T6 (53 ± 11%) ⩾ K (46 ± 9%) while MS smoke ammonia was T6 > T5 > K (statistically significant differences in mean values are shown) (Armitage et al., 2004b). The cigarette with the greatest amount of MS smoke ammonia (T6) was not the cigarette with the highest amount of nicotine mouth retention (T5). Therefore, factors other than ammonia must be involved in controlling nicotine retention in the mouth during smoking.

Also shown in Table 2 are the results of two inhalation studies performed by Armitage et al. (2004b). Nicotine retention at the shallow 75 mL inhalation was 80–88% (no statistically significant difference among the three cigarettes K, T5 and T6). However, in the 500 mL inhalation experiment, nicotine retention for T6 was greater than for the control: T6 (99.6 ± 0.2) > K (99.1 ± 0.5%) ⩾ T5 (98.8 ± 0.6%) (statistically significant differences in mean values are shown). The marginally though statistically significantly differences in the 500 mL inhalation are not considered important in terms of human smoking absorptions of nicotine for several reasons: (1) There is essentially quantitative retention of nicotine at normal inhalation volume. (2) No difference was observed in nicotine uptake into venous blood at any of the inhalation conditions. (3) There was no difference in nicotine retention at shallow inhalations. (4) The now generally recognized phenomenon of smoking compensation (US Department of Health and Human Services October, 2001) and variations in smoking behavior (Baker and Lewis, 2001; Scherer, 1999) would trivialize any marginal though statistically significant variations in nicotine retention cited above.

This data set (Armitage et al., 2004b) and others (Armitage et al., 2004a; Sinclair et al., 1998) provide another key finding: the percent nicotine retention was significantly greater than percent solanesol retention (data not shown in Table 2). Solanesol (**14**) is an endogenous tobacco terpene alcohol that transfers to MS smoke. Because of its relatively large molecular weight, solanesol is non-volatile and is only found in the particles of MS smoke (for leading references, see (Baker, 1999; Seeman et al., 2004)). Therefore, solanesol can only be deposited during smoking by direct deposition of smoke particles (Mechanism A in Fig. 3) (Seeman et al., 2004). That the retention of nicotine is greater than the retention of solanesol during smoking is strong evidence that some of the nicotine evaporates from smoke particles during deep lung inhalation during smoking (Mechanism B in Fig. 3). A similar result was published several years before the Armitage work in a conference proceeding (Sinclair et al., 1998).



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7. The effect of ammonia on the absorption of nicotine by smokers and non-smokers from environmental tobacco smoke

Both smokers and non-smokers can be exposed to environmental tobacco smoke (Air Resources Board, 2005; Gori and Mantel, 1991; Guerin and Jenkins, 1992; Guerin et al., 1992; IARC Working

Table 2
Retention of nicotine by ten male smokers as a function of MS smoke ammonia levels

	Control cigarette (K)	Ammonia-forming ingredients in the reconstituted tobaccos (T5)	Ammonia-forming ingredients applied to the tobacco blend (T6)
FTC tar (mg/cig)	9.6	10.2	9.3
FTC nicotine (mg/cig)	0.67	0.70	0.65
Mainstream smoke ammonia (μg/cig)	16	26	38
Smoking conditions	Nicotine retention (%)[a,b]		
2-s mouth-hold only	46 ± 9*	64 ± 11*,**	53 ± 11**
75 mL inhalation[a,c,d]	88	85	80
500 mL inhalation[a,b,d]	99.1 ± 0.5*	98.8 ± 0.6*	99.6 ± 0.2*,**

Data from Armitage et al. (2004b).[a]

[a] Analytical data, smoke retention data, and statistical significances provided by Armitage et al. (Armitage et al., 2004b).
[b] Comparisons (*pairs of data*) *marked with an* "*" *or a* "**" sign are statistically significantly different $p > 0.05$.
[c] No statistical significance observed for values in this row.
[d] Two-s breath-hold.

Group on the Evaluation of Carcinogenic Risks to Humans, 2004; Jenkins et al., 2000). ETS is formed from sidestream smoke, exhaled mainstream smoke, and some smoke that emanates from the cigarette paper itself during puffing and smolder. ETS, of course, is mixed with the contents of the environmental air. The constituents and concentration of ETS change with time due to reactions, deposition, dilution, and mixing.

In addition, many ETS constituents that are originally in the particles of sidestream smoke evaporate from those particles due to the expansion of the aerosol into the environment and the other factors mentioned above. While >95% of nicotine in MS smoke is in the particles, greater than 95% of nicotine in ETS is in the gas-phase (Baker, 1999; Jenkins et al., 2000; Ogden et al., 1993). This dramatic difference between ETS and MS smoke is due to nicotine's rapid evaporation from sub-micron size smoke particles when these particles expand into larger volumes such as into a room or an outdoor location. Lunell and co-workers among others have demonstrated that, when inhaled, gas-phase nicotine deposits primarily in the mouth (Bergström et al., 1995; Lunell et al., 1996). By analogy, nicotine in ETS will also deposit primarily in the mouth. Indeed, nicotine is present in much lower concentrations in ETS than in MS smoke due to both dilution in the environment (Jenkins et al., 2000) and deposition onto surfaces (Piade et al., 1999). Thus, as explained in the next two paragraphs, nicotine in ETS will act independent of any other gases in ETS including ammonia and, when inhaled from outdoor or room environments, will deposit primarily in the buccal cavity.

Ammonia, a gas at normal temperatures and pressures, evaporates from smoke particles much faster than does nicotine (Seeman et al., 2004). Thus, ammonia in ETS is anticipated to also be nearly exclusively in the gas-phase. The very low concentrations of both ammonia and nicotine in ETS will make any influence on the deposition and retention of either one by the other most unlikely. In fact, ammonia is naturally present is the body, including the mouth and exhaled breath, at concentrations higher than in ETS (Hunt et al., 2002; Hunt et al., 2000).

In conclusion, gases typically act independently of each other with regard to deposition, especially when the constituents in the gases are very dilute as in environmental tobacco smoke. Therefore, if gas phase nicotine at higher concentrations as found in nicotine vapor phase inhalers deposits primarily in the mouth, then nicotine at much lower concentrations as found in ETS will also deposit primarily in the mouth.

8. The non-value of "smoke pH"

By chemical theory, pH measurements are valid only when measured on dilute aqueous solutions. Gases and solids or heterogeneous mixtures of these, with or without an aqueous phase, do not have a pH-value in the rigorous definition of the parameter (Pankow, 2001; Seeman, 2007a; Willems et al., 2006). Tobacco and tobacco smoke, neither being dilute aqueous solutions, do not have pH values. Over the past decades, scientists both within and outside the tobacco industry have measured the pH of aqueous extracts of tobacco smoke (Dixon et al., 2000; Pankow, 2001; Rodgman, 2000; Seeman, 2007a). Some researchers have inserted special electrodes into the smoke stream and have measured pH values, also termed "smoke pH" (see, for example: Brunnemann and Hoffmann, 1974; Callicutt et al., 2006b; Rickert et al., 1982; Sloan and Morie, 1976). The resultant measurements have been termed "smoke pH". There is no reason that these two pH measurement methodologies would lead to identical values, and recent work (Callicutt et al., 2006b) has demonstrated that they, in fact, lead to statistically significantly different pH values for the same cigarettes. The use of "smoke pH" has been seriously criticized (Callicutt et al., 2006b; Pankow, 2001; Seeman, 2007a), and we agree with these criticisms. These measurements can at best provide only an indication of the relative molar quantities of aqueous extractable acids and bases present in either tobacco or smoke. The complex physical nature of tobacco and smoke – both heterogeneous mixtures – combined with their time-dependent nature renders the pH values of very limited value, if any.

The well-known Henderson–Hasselbalch (H–H) equation *correctly* relates the fraction of ionized to unionized species in a dilute aqueous solution to the pH of that solution. The fraction of the three relevant forms of nicotine **1–3** in a dilute aqueous solution can be calculated based on the relationship between the pH of solution and the two pK values of nicotine, one for each nitrogen, as shown in Fig. 5. Because smoke is not a dilute aqueous solution, the relationship shown in Fig. 5 cannot quantitatively calculate, and perhaps not even quantitatively estimate, the distribution of **1–3** in smoke. "Smoke pH" values have been obtained by measuring the pH of an aqueous extract of trapped smoke. Such a measurement reflects the relative molar quantities of aqueous extractable acids and bases in smoke. Not all smoke acids and bases are water soluble (Schmeltz and Hoffmann, 1977; Stedman, 1968) and thus will not be extracted from trapped smoke. The relationship, if any, between the partitioning of nicotine in its various forms in dynamic tobacco smoke aerosol and the pH of an aqueous extract of trapped and aged smoke (or the pH value of an electrode inserted into a smoke stream) is not known.

Some workers mistakenly continue to use the "pH of aqueous extracts of smoke" to quantify the percentage of the forms of nicotine (**1–3**) in smoke. For example, Willems et al. (2006) calculated that "at pH 7.8 about 30% of total nicotine in smoke is present as

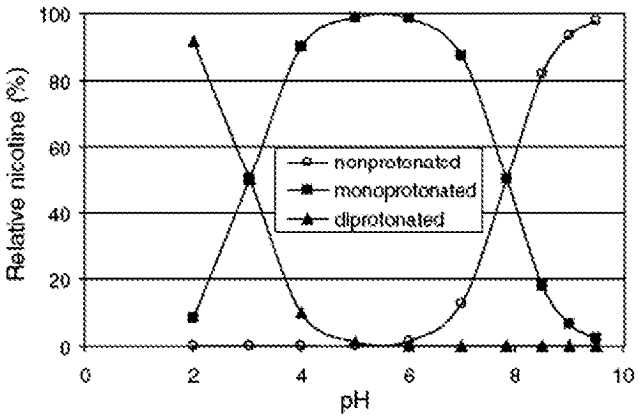

Fig. 1. Chemical structures of nicotine's forms and their percentages as function of pH, ranging from 2 to 9.5. Modified from Hoffmann and Hoffmann (1997).

Fig. 5. This graphic and the above caption are reproduced from Willems et al. (Willems et al., 2006). The original caption refers to a publication by Hoffmann and Hoffmann (Hoffmann and Hoffmann, 1997). The *x*-axis would be more accurately labeled "pH of a dilute aqueous solution". This graph represents the relative proportions of **1–3** in a dilute aqueous solution as a function of pH of the solution. Because smoke is not a dilute aqueous solution, the relationship shown in the figure cannot quantitatively calculate, and perhaps not even estimate, the distribution of **1–3** in smoke aerosol. "Smoke pH" values have been obtained by measuring the pH of an aqueous extract of trapped smoke. Such a measurement reflects the relative molar quantities of aqueous extractable acids and bases in smoke. "Smoke pH" values have also been obtained by placing an electrode into a stream of smoke. In such a measurement, the smoke is expanded in volume, substances may evaporate from the smoke particles, and glass surfaces may capture smoke constituents. The relationship, if any, between the partitioning of nicotine in its various forms in dynamic tobacco smoke aerosol and the pH of an aqueous extract of trapped and aged smoke, and the pH values of an electrode placed in smoke, are not known. See the text for further discussion. Reprinted with permission from (Willems et al., 2006). Copyright (2006) Elsevier.

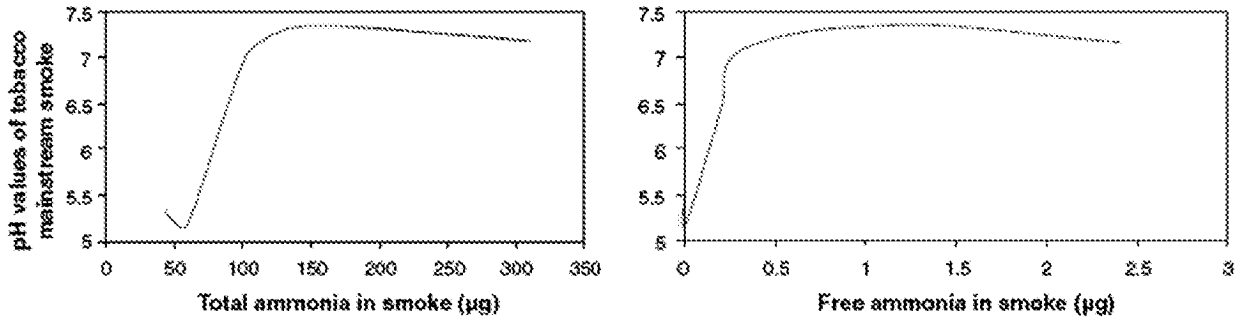

Fig. 2. Effect of total ammonia smoke levels on measured pH value of tobacco mainstream smoke. Modified from Sloan and Morie (1976).

Fig. 6. These two graphs and the above caption are reproduced from Willems et al. (Willems et al., 2006). The original caption shown above refers to a publication by Sloan and Morie (Sloan and Morie, 1976) though the only graph presented in that Sloan and Morie publication is the fraction of free (nonprotonated) ammonia as a function of "smoke pH," not "free ammonia in smoke (µg)" (Willems et al., 2006). Two different electrodes were used by Sloan and Morie in 1976 (Markson combination electrode for "smoke pH" and Orion electrode No. 95-10 for nonprotonated ammonia). In these measurements, the MS smoke aerosol was passed over one or the other electrode. Complicating these measurements is the fact that most of ammonia in fresh MS smoke is in the particles, not in the gas phase, and there is rapid evaporation of ammonia from these smoke particles as the aerosol expands, e.g., into the chamber in which the electrode is placed. For these reasons and other reasons discussed in the text and in the caption for Fig. 5, neither "total ammonia in smoke" nor "free ammonia in smoke" can quantitatively predict, and perhaps not even estimate, "smoke pH", nor the converse. Reprinted with permission from (Willems et al., 2006). Copyright (2006) Elsevier.

free-base" non-protonated nicotine (**1**). Willems et al. (2006) also presented two graphs (reproduced in Fig. 6) dealing with "free ammonia" (i.e., non-protonated ammonia, NH_3) and total ammonia (i.e., the sum of NH_3 and ammonium ion, NH_4). The graphs were used to demonstrate "the effect of ammonia on smoke pH. . . showing that among different tobacco products the level of total and free ammonia in smoke proved to be positively associated with the pH of the smoke" (Willems et al., 2006). It is valid that, holding all MS smoke constituents constant but increasing the amount of ammonia in MS smoke, the pH of aqueous extracts of the smoke may increase. However, the graphs in Fig. 6 cannot be general or predictive for all cigarettes smoked under all smoking protocols. Factors such as the amount and relative concentrations of other smoke constituents including possible buffers must be taken into consideration. Again, the quantitative application of the H–H equation to ammonia in smoke is inappropriate. The relationship, if any, between the partitioning of ammonia in its two forms (ammonia and ammonium) in dynamic tobacco smoke aerosol and the pH of an aqueous extract of trapped and aged smoke (or the pH value of an electrode inserted into a smoke stream) is not known. In addition, and more fundamentally valid, the graphs in Fig. 6 cannot be used to calculate the effect of ammonia on "smoke pH" nor can it relate the fraction of non-protonated ammonia in smoke with "smoke pH". The relationships, if any, between the concentration of ammonia in smoke, the acid–base properties of smoke aerosol, and the pH of dilute aqueous extracts of smoke are not known.

There is another serious flaw in the use of "smoke pH" for either quantitative or qualitative purposes which has only recently been reported, that is the temporal qualities of MS smoke (Seeman, 2007a). MS smoke is a dynamic aerosol of heterogeneous particles suspended in gases. The chemical composition of MS smoke changes with time as does the absolute and relative concentration of smoke's constituents in the gases and particles (Baker, 1999; Baker and Dixon, 2006; Seeman, 2007a; Seeman et al., 2004). For example, during smoking, many time-dependent processes will occur, some of which are as follows: water soluble gas constituents will deposit in the mouth and upper respiratory tract; most of smoke's particles will, upon inhalation, transfer to the lung though some of the largest particles may deposit in the mouth; evaporation of smoke constituents originally in the particles will occur, the rate being dependent on both their concentration in the particles and their volatility; particle size will change, and water from the lungs will deposit onto the particles; particles will deposit on the lung–blood interface; and smoke aerosol will be exhaled (Baker, 1999; Ingebrethsen, 2006; Ingebrethsen and Lyman, 2002; Pankow, 2001; Seeman, 2007a; Seeman et al., 2004). Hence, it is extremely unlikely that any single time-independent parameter (e.g., "smoke pH" or "smoke pH_{eff}" or the fraction of non-protonated nicotine in smoke, termed α_{fb}) can adequately describe the time-dependent properties of MS smoke aerosol. To date, in spite of a large amount of experimental data on "smoke pH" (Rodgman, 2000), this parameter has not been shown to be a useful or predictive parameter. We conclude that "smoke pH" and "smoke pH_{eff}" and α_{fb} are not likely to be found to be useful or predictive of nicotine's bioavailability to the smoker.

"Sidestream smoke pH" has also been discussed in the literature. For example, Willems et al. (2006) stated that "the pH-value of SS smoke averages that of MS smoke (pH of 6.7–7.5), so that up to30% of total nicotine in SS smoke is present in its free-base form. Obviously, the addition of ammonia to tobacco also increases the (free-base) nicotine level in SS smoke" (Willems et al., 2006). As discussed in detail in the paragraphs immediately above, the measured pH values of trapped smoke should not be used to quantify the fractions of the forms of nicotine (**1**–**3**) in smoke, whether it be mainstream smoke or sidestream smoke or environmental tobacco smoke. By definition, sidestream smoke is the smoke stream that issues primarily from the burning end of the cigarette but also, to a lesser extent, through the paper or filter, generally during smolder. Sidestream smoke immediately diffuses into the surrounding environment, be it a room or the outside, and becomes part of environmental tobacco smoke. During this process, the mass of the smoke expands in volume enormously. One consequence of that expansion is that the nicotine, which is mostly (>99%) in the particles of mainstream smoke, is almost entirely (>95%) in the gas phase of ETS (Baker, 1999; Ogden et al., 1993). Only the non-protonated form of nicotine (**1**) can exist in the gas phase. Thus, >95% of nicotine in ETS is non-protonated. It is therefore unlikely that ammonia in smoke can enhance the percent of nicotine in the gas phase of ETS, as implied in the literature (Willems et al., 2006). For more discussion of deposition of nicotine in ETS by humans and related topics, see the discussion in Section 7.

Given the conclusions above, and pending any experimental results that demonstrate some utility, we conclude that it is not in the interest of tobacco scientists or the public health community to focus attention on "smoke pH" and "smoke pH_{eff}" and α_{fb}. Our conclusion contrasts with a 2007 report by the World Health Organization (World Health Organization, 2007) and recent reviews

(Henningfield et al., 2004b; Pankow, 2001; Willems et al., 2006). The scientifically unsupportable attention on pH can only distract attention and misdirect resources from discovering more meaningful insights that can better lead to reduced harm to the smoking population.

9. Risk assessment

As discussed in the sections above, these laboratory and human smoking studies are inconsistent with the hypotheses that ammonia or ammonia-releasing ingredients[1] in tobacco or ammonia in mainstream smoke increases the amount or total rate of nicotine transfer to the arterial bloodstream or brains of smokers during smoking. However, there are no experiments that have quantified the effect of varying concentrations or varying amounts of ammonia in MS smoke on the rate or amount of nicotine reaching the arterial blood or brains of smokers. Experiments along these lines can be envisioned, and while they are complex, there are no fundamental principles which would prevent their execution. However, even if such experiments are performed and show no ammonia effect, they would not unambiguously resolve the potential public health concerns. This is due to the huge amount of publicity, attention and public health implications that have been engendered by these hypotheses made over such a long period of time.

For a conservative risk assessor, the uncertainty of the consequences of the integration of all the indirect experimental results (negative results) will still only lead to continued ambiguity. For the ammonia-hypothesis controversy to be unambiguously resolved, an epidemiological study would be required which would compare disease rates and quitting rates (a measure of addiction and health consequences (IARC Working Group, 2007)) in smokers of cigarettes that contain various amounts of ammonia-forming ingredients. Such groups of smokers may exist. For example, some of Philip Morris USA's cigarettes sold in the United States contain ammonia-forming ingredients whereas other cigarettes sold in the United States may not contain such ingredients. Comparison of these groups could provide meaningful results. An epidemiological comparison of cigarettes sold in different countries will be subject to significant confounders and is therefore less preferable. Two standard types of epidemiological could be performed, namely the case-control and prospective studies. While the number of subjects required for these types of studies cannot be determined prior to doing a sensitivity analysis, it would appear that this type of study may be practicable.

It should be noted that, while not epidemiological data, sales and market share data do not correlate with ammonia levels in tobacco and smoke. There are many US commercial cigarettes, including brands sold by Philip Morris USA, which have similar ammonia yields but have far, far less sales and market share than do the market leaders (Counts et al., 2007; Maxwell, 2007; Taylor et al., 2000). If ammonia were a truly important component in a cigarette's commercial success and/or to its addictive potential, then the competitiveness as judged by the market place would be quite different. In other words, there are many brands of commercial cigarettes having essentially the same ammonia levels as sales leading cigarettes but have far less market share.

A reviewer has asked us "which assumptions would be overly conservative and not tenable on the basis of the available evidence." Some people would argue that, when it comes to tobacco and the public health, any scientific data that indicates that an ingredient does not increase the risk of disease to smokers is irrelevant and may even be counterproductive to the public health if the use of such an ingredient helps sell cigarettes (see, for example: Gray, 2006; Gray and Kozlowski, 2003; Henningfield et al., 2004a; Rabinoff et al., 2007). The public health goals of preventing initiation and increasing smoking cessation respond to the enormous health burden caused by smoking. In order to achieve such public health goals, we ask: is it necessary and even appropriate to continue to focus attention on ammonia-forming ingredients, seemingly on the basis of science (Henningfield and Zeller, 2002; Kessler et al., 1997; Samet and Burke, 2001; World Health Organization, 2007), when the scientific data is not supportive of such attention? We note the long-standing "principle that resources should be directed towards the testing and evaluation of those substances with the greatest potential to produce [and increase] human risk and away from those with a low potential for risk" (Munro et al., 1996, 1999). We have presented in this review a wide body of experimental data which speaks for itself regarding the hypotheses that ammonia enhances nicotine bioavailability.

10. Conclusions

Cigarette smoking is addictive and causes disease. Because nicotine is considered to be the addictive agent in cigarette smoke, knowledge of the chemistry and processes associated with the transfer of nicotine from tobacco to smoke and to the CNS of smokers is of great importance. Any hypothesis that the transfer of nicotine from tobacco to smoke or nicotine bioavailability to the CNS is enhanced by tobacco ingredients, e.g., ammonia-forming ingredients, needs to be carefully evaluated using robust experimentation.

For occupational exposure to ammonia at levels which are, in general, much higher than found in tobacco smoke, little or no difference in any of the measured biological endpoints has been observed. No evaluations of ammonia toxicity in the context of smoking are currently available. Regarding the possibility of toxicological interactions due to the thousands of other smoke constituents, any extrapolation to human smoking must be done with extreme caution.

To date, there has been no direct experimental data reported on the effect of ammonia-releasing compounds in tobacco or ammonia in MS smoke on the rate or amount of nicotine transfer to the arterial bloodstream or to the rate and amount of nicotine reaching the brains of smokers during smoking. Calls for these more definitive types of experiments have been made in the literature (Henningfield et al., 2004b; Seeman, 2007a; World Health Organization, 2007). We are fully in agreement that such experiments would be enormously valuable. In the absence of such experiments, researchers have relied on indirect studies which have shed light on segments, or subsets, of the total tobacco-to-brain sequence. These segments include: (a) the transfer of nicotine from tobacco to smoke; (b) the ability of machine-smoking methods to quantify all the nicotine in MS smoke; (c) the location of deposition of nicotine; (d) the total rate and amount of nicotine deposition and retention by human smokers; (e) the rate of nicotine transfer from the buccal cavity to the bloodstream and from the deep lung through the lung–blood interfaces; (f) deposition and retention mechanisms of nicotine; and (g) transport mechanisms of nicotine through the lung–blood interfaces.

The results of a large variety of experiments discussed in the sections above, individually and in total, are inconsistent with the hypotheses that ammonia or ammonia-releasing ingredients[1] in tobacco or ammonia in mainstream smoke increases the amount or total rate of nicotine transfer to the arterial bloodstream or brains of smokers during smoking. The bases for this conclusion will now be summarized.

A wide range of experimental data has been obtained over a 30-year period examining the ability of a Cambridge filter pad to trap MS smoke nicotine during machine-smoking. These studies, individually and taken together, demonstrate that the vast majority

(>99%) of the nicotine in MS smoke of both commercial and experimental cigarettes is captured by the Cambridge filter pad and quantified by machine-smoking methods. Recent studies have demonstrated that the presence of ammonia-forming ingredients in tobacco or additional ammonia in MS smoke do not interfere with the FTC and ISO methods from quantifying ⩾99% of the nicotine in MS smoke.

Nicotine is relatively thermally stable, decomposing in air at temperatures >300 °C and in inert atmosphere at temperatures >600 °C. Pyrolysis and thermal analysis studies of non-protonated nicotine (**1**) and various protonated nicotine carboxylic acid salts **2** and **3** as found in tobacco indicate that **1** and **2** transfer nicotine to the aerosol upon heating with similar efficiencies and at temperatures well below the decomposition temperatures of the nicotine ring system. Temperature distributions of the gases and the coal within a burning and puffing cigarette indicate that most of the nicotine volatilizes from the hot zones before it experiences temperatures ⩾ca. 250 °C. Thus, nicotine and protonated nicotine transfer nicotine to the aerosol at temperatures below the temperatures required to destroy the nicotine ring system. Nicotine and its salts transfer nicotine to smoke with little decomposition and without the need for added bases such as ammonia. These thermolysis results are inconsistent with the hypothesis that ammonia or bases are either necessary or enhance the transfer of nicotine from tobacco to the gas phase during heating of its protonated forms, i.e., during smoking.

Regarding the effect of ammonia on the transfer of nicotine from tobacco to smoke, only one study has been reported that has all the complete set of required experimental data, the theoretical underpinning, and statistical analyses (Callicutt et al., 2006b). This study demonstrates no increase in the amount of nicotine transfer from tobacco to MS smoke with an increase in ammonia-forming ingredients in tobacco, with an increase in tobacco soluble ammonia, or with an increase in MS smoke ammonia. These results are in full agreement with the model pyrolysis/thermal studies of nicotine and nicotine salts.

Greater than 90%, and in some experiments, >99%, of the nicotine *inhaled* during smoking is retained by human smokers. Thus, there is little room for increasing the amount of nicotine absorbed during smoking by additives – other than increasing the amount of nicotine in smoke. As discussed above, ammonia-forming ingredients do not increase the transfer of nicotine from tobacco to smoke. No increases in nicotine levels in venous blood of human smokers were observed for cigarettes having increased ammonia in the MS smoke.

There has been some confusion in the literature regarding the difference in the role of the forms of nicotine (**1**–**3**) and nicotine bioavailability to the CNS as these relate to nicotine's deposition and absorption by the smoker. For example, it was recently concluded that "The absorption rate of nicotine depends therefore on the form (free base, protonated) in which nicotine is presented to the buccal-pulmonary epithelial tissue. Similarly, the absorption rate of nicotine and the amount of nicotine absorbed is smoke pH dependent" (Willems et al., 2006). First, "smoke pH" is not considered to be a useful parameter (see the text and the conclusions below). Second, specific instances have been documented in which there are acid–base effects on nicotine absorption in the oral cavity and dermal tissue (Baker and Dixon, 2006; Hukkanen et al., 2005; Nair et al., 1997). In each of these cases, the buffering capacity of the system was experimentally overwhelmed due to the amount of acidic or basic exogenous materials.

In contrast, the acid–base nature of tobacco smoke is unlikely to affect nicotine absorption from the respiratory epithelium including the lung (Benowitz, 1998a; Dixon et al., 2000; Gourlay and Benowitz, 1997; Karan et al., 2003; Seeman, 2007a; Stitzer and Wit, 1998; Surgeon General, 1988; Willems et al., 2006) for several reasons. (1) The amount and concentration of nicotine and other smoke constituents per puff is small. (2) This nicotine is spread over the huge surface area of the lung. (3) The lung has a large buffer capacity that will not be overwhelmed by tobacco smoke. (4) There is some evidence that nicotine can be retained within the lung–blood interfaces for one or more minutes, thereby leveling out any theoretical ammonia effects. Thus, the acid–base character of smoke particles will not control the rate or amount of nicotine through the lung–blood interfaces by passive transport.

There is evidence that protonated nicotine may transfer through the lung–blood interface by active transfer by using the choline transporter. In fact, researchers are utilizing these active transport systems in the brain to develop drugs that will interfere with this process, e.g., inhibition of nicotine addiction and enhancing smoking cessation. Active transport of nicotine is a generally widespread phenomenon with the only reported exceptions being by the dermal and buccal routes. This active transport system for nicotine would not be significantly impacted by ammonia in smoke. In any future discussions relating to nicotine transport in biological systems, the role of active as well as passive transport need to be considered.

"Smoke pH" and "smoke pH_{eff}" and the fraction of non-protonated nicotine in smoke (sometimes referred to as α_{fb}) of commercial cigarettes are not useful, practical smoke parameters for providing understanding or predictability of nicotine bioavailability to smokers.

Nicotine in ETS is present nearly exclusively in the gas phase. Gas phase nicotine deposits primarily in the buccal cavity. Hence, ammonia in ETS is unlikely to affect ETS nicotine bioavailability to exposed smokers and non-smokers.

In summary, the extant experimental data is inconsistent with either ammonia-releasing compounds in commercial cigarettes or ammonia in MS smoke increasing the total rate or amount of nicotine reaching the arterial blood or brains of smokers. There are no experiments that have quantified the effect of varying concentrations or varying amounts of ammonia in MS smoke on the rate or amount of nicotine reaching the arterial blood or brains of smokers. Epidemiological studies which compare disease rates and quitting rates in smokers of cigarettes that contain various amounts of ammonia-forming ingredient could be performed. Such epidemiological studies may well be required before the public health concerns regarding ammonia and nicotine will finally be settled.

Conflict of interest statement

The authors (JIS and RAC) were employees of Philip Morris USA for many years and both consult for both Philip Morris USA and Philip Morris Products S.A. Richard A. Carchman has served in the past as a witness for Philip Morris USA in various litigations. This paper is based, in part, on research now published in several peer-reviewed journals and cited herein under a contract to SaddlePoint Frontiers. Neither author received funds from any external source in the writing of this manuscript.

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